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MICROSATELLITE MARKERS FOR PLANTS OF THE SPECIES TRITICUM AESTIVUM AND TRIBE TRITICEAE AND THE USE OF SAID MARKERS

The invention relates to novel genetic markers for wheats (Triticum aestivum L.) and closely related species (Tribus Triticeae) and to the use of said markers.

The most widely spread, known, DNA-based genetic markers are the so-called restriction fragment length polymorphisms (RFLP) markers. For using these markers, genomic DNA is digested with restriction enzymes, separated on agarose gels and transferred to nylon membranes (Southern Blot). Specific fragments are detected by hybridization with radioactively labeled DNA probes. When mutations occur in the region of the restriction enzymes used or when smaller deletions/insertions occur, polymorphisms between different lines are found, which are passed on stably and mostly codominantly. The use of RFLP markers in hexaploid cultivated wheat is possible only to a limited extent, since only very little polymorphism is detected in wheat in this manner.

It has already been described that microsatellite markers detect significantly more polymorphism between different wheat lines than do RFLP markers. This can be attributed particularly to the occurrence of multiple alleles per locus (Röder et al., Mol. Gen. Genet. (1995) 246, 327 - 333). Moreover, it is known that microsatellite markers have the advantage that they can be detected by way of PCR and that therefore large amounts of samples can be analyzed more easily.

It is an object of the invention to provide novel microsatellite markers for the genetic analysis of plants of the Triticum aestivum species, which markers are distinguished by a degree of DNA polymorphism, which is higher than that of other molecular probes, that have been developed previously for the wheat genome.

This objective is accomplished by claims 1 to 10. The inventive markers are based on the amplification of certain hypervariable genome sections, the so-called microsatellites, with the help of their polymerase chain reaction (PCR). For specific amplification, two primers, in each to the case left and the right in the flanking sequences, are required for each microsatellite locus. On the average, these primers are 20 ± 3 bases long and are defined by their sequences. In principle, a microsatellite marker is a sequence tagged site (STS), which is defined by two specific primers. These primers flank, in each case to the left and the right, a so-called microsatellite sequence. A microsatellite sequence is defined as a tandem repetitive repetition of a di-, tri- or tetranucleotide sequence, for example $(GA)_n$, in which $n \geq 10$. Composite microsatellite sequences also occur, such as $(GT)_n(AT)_n$, as well as imperfect sequences, in which individual bases are mutated, such as $(GA)_nCA(GA)_n$. Among various lines and varieties, there is variation in the number of repeats at a certain locus. After amplification of the microsatellites, this leads, by means of the specific primers in the flanking sequences, to PCR products of different length and, with that, to polymorphisms. These polymorphisms are passed on stably and can therefore be used as genetic markers. In some cases, null alleles (no visible fragment) also occur, when there are mutations within the binding site for the primers.

The separation and detection of the PCR products obtained can be carried out with different technical variants. For separating the fragments, highly resolving agarose gels, native polyacrylamide gels or denaturing polyacrylamide gels (= sequencing gels) can be used. Depending on the separation system, fragments are detected using ethidium bromide staining, silver staining or, after labeling the PCR

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fragments radioactively, using autoradiography. A further, very effective variation for separation and detection consists of the use of an automatic sequencer with dye- or fluorescence-labeled primers. For this purpose, it is necessary to synthesize a dye- or fluorescence-labeled primer from each microsatellite primer pair. PCR amplification results in a labeled product, which can be detected by the sequencing equipment. At the same time, dye- or fluorescence-labeled size standards are also separated for each sample in the same track. After that, special software enable the absolute size of each fragment, which has been separated, to be calculated and, with that, also permits fragments from different gel runs to be compared. With this method, several hundred samples can be analyzed largely automatically in a day.

Pursuant to the invention, microsatellite markers are made available, which contain the following primer pairs with assigned microsatellite sequences or a number thereof and amplify the loci of all chromosomes of the wheat genome and therefore find use for gene marking.

WMS Number	WMS Primer Left	WMS Primer Right	Length (bp in "cs")	Repeat Type	Annealing Temperature
WMS052	5'CTA TGA GGC GGA GGT TGA AG 3' (SEQ. ID NO. 1)	5'TGC GGT GCT CTT CCA TTT 3' (SEQ. ID NO. 2)	150	GTimp	60 °C
WMS055	5'GCA TCT GGT ACA CTA GCT GCC 3' (SEQ. ID NO. 3)	5'TCA TGG ATG CAT CAC ATC CT 3 (SEQ. ID NO. 4)	127	CTimp	60 °C
WMS057	5'TCG ATT CTG AAA GGT TCA TCG 3' (SEQ. ID NO. 5)	5'CGA TCA AGT AGT TGA AAG CGC 3' (SEQ. ID NO. 6)	224	AAAAAimp	60 °C
WMS058	5'TCT GAT CCC GTG AGT GTA ACA 3' (SEQ. ID NO. 7)	5'GAA AAA AAT TGC ATA TGA GCC C 3' (SEQ. ID NO. 8)	118	CA	60 °C
WMS060	5'TGT CCT ACA CGG ACC ACG T 3' (SEQ. ID NO. 9)	5'GCA TTG ACA GAT GCA CAC G 3' (SEQ. ID NO. 10)	211	CA	60 °C
WMS063	5'TCG ACC TGA TCG CCC CTA 3' (SEQ. ID NO. 11)	5'CGC CCT GGG TGA TGA ATA GT 3' (SEQ. ID NO. 12)	271	GAA,CA,TA	60 °C
WMS067	5'ACC ACA CAA ACA AGG TAA GCG 3' (SEQ. ID NO. 13)	5'CAA CCC TCT TAA TTT TGT TGG G 3' (SEQ. ID NO. 14)	85	CA	60 °C
WMS068	5'AGG CCA GAA TCT GGG AAT G 3' (SEQ. ID NO. 15)	5'CTC CCT AGA TGG GAG AAG GG 3' (SEQ. ID NO. 16)	182	GA	60 °C
WMS070	5'AGT GGC TGG GAG AGT GTC AT 3' (SEQ. ID NO. 17)	5'GCC CAT TAC CGA GGA CAC 3' (SEQ. ID NO. 18)	194	GT	60 °C
WMS071	5'GGC AGA GCA GCG AGA CTC 3' (SEQ. ID NO. 19)	5'CAA GTG GAG CAT TAG GTA CAC G 3' (SEQ. ID NO. 20)	128	GT	60 °C
WMS077	5'ACA AAG GTA AGC AGC ACC TG 3' (SEQ. ID NO. 21)	5'ACC CTC TTG CCC GTG TTG 3' (SEQ. ID NO. 22)	153	CA,GA	55 °C
WMS082	5'ACG TTA GAA GGT GCA ATG GG 3' (SEQ. ID NO. 23)	5'AGT GGA TGC ACC GAC TTT G 3' (SEQ. ID NO. 24)	152	GT,GAimp	60 °C
WMS088	5'CAC TAC AAC TAT GCG CTC GC 3' (SEQ. ID NO. 25)	5'TCC ATT GGC TTC TCT CTC AA 3' (SEQ. ID NO. 26)	121	GT	60 °C
WMS095	5'GAT CAA ACA CAC ACC CCT CC 3' (SEQ. ID NO. 27)	5'AAT GCA AAG TGA AAA ACC CG 3' (SEQ. ID NO. 28)	121	CA	60 °C
WMS099	5'AAG ATG GAC GTA TGC ATC ACA 3' (SEQ. ID NO. 29)	5'GCC ATA TTT GAT GAC GCA TA 3' (SEQ. ID NO. 30)	119	CA	60 °C
WMS102	5'TCT CCC ATC CAA CGC CTC 3' (SEQ. ID NO. 31)	5'TGT TGG TGG CTT GAC TAT TG 3' (SEQ. ID NO. 32)	143	CT	60 °C
WMS106	5'CTG TTC TTG CGT GGC ATT AA 3' (SEQ. ID NO. 33)	5'AAT AAG GAC ACA ATT GGG ATG G 3' (SEQ. ID NO. 34)	139	GA	60 °C
WMS107	5'ATT AAT ACC TGA GGG AGG TGC 3' (SEQ. ID NO. 35)	5'GGT CTC AGG AGC AAG AAC AC 3' (SEQ. ID NO. 36)	195	CT	60 °C
WMS108	5'CGA CAA TGG GGT CTT AGC AT 3' (SEQ. ID NO. 37)	5'TGC ACA CTT AAA TTA CAT CCG C 3' (SEQ. ID NO. 38)	132	GTimp	60 °C
WMS111	5'TCT GTA GGC TCT CTC CGA CTG 3' (SEQ. ID NO. 39)	5'ACC TGA TCA GAT CCC ACT CG 3' (SEQ. ID NO. 40)	205	CT,GT	55 °C
WMS112	5'CTA AAC ACG ACA GCG GTG G 3' (SEQ. ID NO. 41)	5'GAT ATG TGA GCA GCG GTC AG 3' (SEQ. ID NO. 42)	101	CTimp	55 °C
WMS113	5'ATT CGA GGT TAG GAG GAA GAG G 3' (SEQ. ID NO. 43)	5'GAG GGT CGG CCT ATA AGA CC 3' (SEQ. ID NO. 44)	148	GT	60 °C
WMS114	5'ACA AAC AGA AAA TCA AAA CCC G 3' (SEQ. ID NO. 45)	5'ATC CAT CGC CAT TGG AGT G 3' (SEQ. ID NO. 46)	206 (177)	GA	60 °C
WMS118	5'GAT GTT GCC ACT TGA GCA TG 3' (SEQ. ID NO. 47)	5'GAT TAG TCA AAT GGA ACA CCC C 3' (SEQ. ID NO. 48)	110	CA	60 °C
WMS119	5'TGA CTA ACA TCC TTT GTC ACG C 3' (SEQ. ID NO. 49)	5'CAT GTC TCA ACC ACC CAC AG 3' (SEQ. ID NO. 50)	181	GTimp	55 °C

	Forward primer	SEQ ID	Reverse primer	SEQ ID	Size	Motif	Temp
WMS120	5' GAT CCA CCT TCC TCT CTC TC 3'	(SEQ. ID NO. 51)	5' GAT TAT ACT GGT GCC GAA AC 3'	(SEQ. ID NO. 52)	139	CT,CA	55 °C
WMS121	5' TCC TCT ACA AAC AAC ACA CAC AC 3'	(SEQ. ID NO. 53)	5' CTC GCA ACT AGA GGT GTA TG 3'	(SEQ. ID NO. 54)	143	CA	50 °C
WMS122	5' GGG TGG GAG AAA GGA GAT G 3'	(SEQ. ID NO. 55)	5' AAA CCA TCC TCC ATC CTG G 3'	(SEQ. ID NO. 56)	149	CT,CA	60 °C
WMS124	5' GCC ATG GCT ATC ACC CAG 3'	(SEQ. ID NO. 57)	5' ACT GTT CGG TGC AAT TTG AG 3'	(SEQ. ID NO. 58)	213	CT,GTimp	60 °C
WMS126	5' CAC ACG CTC CAC CAT GAC 3'	(SEQ. ID NO. 59)	5' GTT GAG TTG ATG CGG GAG G 3'	(SEQ. ID NO. 60)	196	CA	60 °C
WMS128	5' AGC ACA TTT TAA CAC AGA TA 3'	(SEQ. ID NO. 61)	5' ATC TGT GAA ATT TTG AAA AC 3'	(SEQ. ID NO. 62)	176	CA	50 °C
WMS129	5' TCA GTG GGC AAG CTA CAC AG 3'	(SEQ. ID NO. 63)	5' AAA ACT TAG TAG CCG CGT 3'	(SEQ. ID NO. 64)	221	GTimp	55 °C
WMS130	5' AGC TCT GCT TCA CGA GGA AG 3'	(SEQ. ID NO. 65)	5' CTC CTC TTT ATA TCG CGT CCC 3'	(SEQ. ID NO. 66)	113	GT	60 °C
WMS131	5' AAT CCC CAC CGA TTC TTC TC 3'	(SEQ. ID NO. 67)	5' AGT TCG TGG GTC TCT GAT GG 3'	(SEQ. ID NO. 68)	131	CT	60 °C
WMS132	5' TAC CAA ATC GAA ACA CAT CAG G 3'	(SEQ. ID NO. 69)	5' CAT ATC AAG GTC TCC TTC CCC 3'	(SEQ. ID NO. 70)	119	GA,GAA	60 °C
WMS133	5' ATC TAA ACA AGA CGG CGG TG 3'	(SEQ. ID NO. 71)	5' ATC TGT GAC AAC CGG TGA GA 3'	(SEQ. ID NO. 72)	118	CT	60 °C
WMS134	5' CAT GGA ACT TAG ACA GAA TTG 3'	(SEQ. ID NO. 73)	5' CAG TAC TTG GTA CTG AAC AGG 3'	(SEQ. ID NO. 74)	111	CA	60 °C
WMS135	5' TGT CAA CAT CGT TTT GAA AAG G 3'	(SEQ. ID NO. 75)	5' ACA CTG TCA ACC TGG CAA TG 3'	(SEQ. ID NO. 76)	143	GA	55 °C
WMS136	5' GAC AGC ACC TTG CCC TTT G 3'	(SEQ. ID NO. 77)	5' CAT CGG CAA CAT GCT CAT C 3'	(SEQ. ID NO. 78)	296	CT	60 °C
WMS140	5' ATG GAG ATA TTT GGC CTA CAA C 3'	(SEQ. ID NO. 79)	5' CTT GAC TTC AAG GCG TGA CA 3'	(SEQ. ID NO. 80)	251	CT	55 °C
WMS144	5' TTT GCT GTG GTA CGA AAC ATA C 3'	(SEQ. ID NO. 81)	5' ACT CAC AAA TGT CTA ATA AAA C 3'	(SEQ. ID NO. 82)	200	GT	50 °C
WMS146	5' CCA AAA AAA CTG CCT GCA TG 3'	(SEQ. ID NO. 83)	5' CTC TGG CAT TGC TCC TTG G 3'	(SEQ. ID NO. 84)	162	GAimp	60 °C
WMS148	5' GTG AGG CAG CAA GAG AGA AA 3'	(SEQ. ID NO. 85)	5' CAA AGC TTG ACT CAG ACC AAA 3'	(SEQ. ID NO. 86)	163	CA	60 °C
WMS149	5' CAT TGT TTT CTG CCT CTA GCC 3'	(SEQ. ID NO. 87)	5' CTA GCA TCG AAC CTG AAC AAG 3'	(SEQ. ID NO. 88)	161	GA	55 °C
WMS153	5' GAT CTC GTC ACC CGG AAT TC 3'	(SEQ. ID NO. 89)	5' TGG TAG AGA AGG ACG GAG AG 3'	(SEQ. ID NO. 90)	188	GA	60 °C
WMS154	5' TCA CAG AGA GAG AGG GAG GG 3'	(SEQ. ID NO. 91)	5' ATG TGT ACA TGT TGC CTG CA 3'	(SEQ. ID NO. 92)	102	GA	55 °C
WMS155	5' CAA TCA TTT CCC CCT CCC 3'	(SEQ. ID NO. 93)	5' AAT CAT TGG AAA TCC ATA TGC C 3'	(SEQ. ID NO. 94)	141	CT	60 °C
WMS156	5' CCA ACC GTG CTA TTA GTC ATT C 3'	(SEQ. ID NO. 95)	5' CAA TGC AGG CCC TCC TAA C 3'	(SEQ. ID NO. 96)	277	GT	60 °C
WMS157	5' GTC GTC GCG GTA AGC TTG 3'	(SEQ. ID NO. 97)	5' GAG TGA ACA CAC GAG GCT TG 3'	(SEQ. ID NO. 98)	106	CT	60 °C
WMS159	5' GGG CCA ACA CTG GAA CAC 3'	(SEQ. ID NO. 99)	5' GCA GAA GCT TGT TGG TAG GC 3'	(SEQ. ID NO. 100)	192	GT	60 °C
WMS160	5' TTC AAT TCA GTC TTG GCT TGG 3'	(SEQ. ID NO. 101)	5' CTG CAG GAA AAA AGT TAC ACC C 3'	(SEQ. ID NO. 102)	184	GA	60 °C
WMS161	5' GAT CGA GTG ATG GCA GAT GG 3'	(SEQ. ID NO. 103)	5' TGT GAA TTA CTT GGA CGT GG 3'	(SEQ. ID NO. 104)	154	CT	60 °C
WMS162	5' AGT GGA TCG ACA AGG CTC TG 3'	(SEQ. ID NO. 105)	5' AGA AGA AGC AAA GCC TTC CC 3'	(SEQ. ID NO. 106)	208	CA	60 °C

Name	Forward primer		Reverse primer		Size	Repeat	Temp
WMS163	5' ACC TCG ACA GAC CTG GTA CG 3'	(SEQ. ID NO. 107)	5' GTC TTT GTC GTC ACC CGA TGG AC 3'	(SEQ. ID NO. 108)	127	CT	55 °C
WMS164	5' ACA TTT CTC CCC CAT CGT C 3'	(SEQ. ID NO. 109)	5' TTG TAA ACA AAT CGC ATG CG 3'	(SEQ. ID NO. 110)	120	CT	55 °C
WMS165	5' TGC AGT GGT CAG ATG TTT CC 3'	(SEQ. ID NO. 111)	5' CTT TTC TTT CAG ATT GCG CC 3'	(SEQ. ID NO. 112)	199	GA	60 °C
WMS169	5' ACC ACT GCA GAG AAC ACA TAC G 3'	(SEQ. ID NO. 113)	5' GTG CTC TGC TCT AAG TGT GGG 3'	(SEQ. ID NO. 114)	196	GA	60 °C
WMS174	5' GGG TTC CTA TCT GGT AAA TCC C 3'	(SEQ. ID NO. 115)	5' GAC ACA CAT GTT CCT GCC AC 3'	(SEQ. ID NO. 116)	173	CT	55 °C
WMS179	5' AAG TTG AGT TGA TGC GGG AG 3'	(SEQ. ID NO. 117)	5' CCA TGA CCA GCA TCC ACT C 3'	(SEQ. ID NO. 118)	181	GT	55 °C
WMS180	5' ATC CGC CTA AGG AAT AGT GT 3'	(SEQ. ID NO. 119)	5' GAT CGC ACG GGA GAG A G 3'	(SEQ. ID NO. 120)	84	CT	50 °C
WMS181	5' TCA TTG GTA ATG AGG AGA GA 3'	(SEQ. ID NO. 121)	5' GAA CCA TTC ATG TGC ATG TC 3'	(SEQ. ID NO. 122)	135	GA	50 °C
WMS182	5' TGA TGT AGT GAG CCC ATA GGC 3'	(SEQ. ID NO. 123)	5' TTG CAC ACA GCC AAA TAA GG 3'	(SEQ. ID NO. 124)	165	CT	60 °C
WMS186	5' GCA GAG CCT GGT TCA AAA AG 3'	(SEQ. ID NO. 125)	5' CGC CTC TAG CGA GAG CTA TG 5'	(SEQ. ID NO. 126)	140	GA	60 °C
WMS189	5' AGG AGC AGC GGA ACG AAC 3'	(SEQ. ID NO. 127)	5' AGA AAT ACG GAA ACC CAC CC 3'	(SEQ. ID NO. 128)	117	CA	55 °C
WMS190	5' GTG CTT GCT GAG CTA TGA GTC 3'	(SEQ. ID NO. 129)	5' GTG CCA CGT GGT ACC TTT G 3'	(SEQ. ID NO. 130)	>201	CT,GT	60 °C
WMS191	5' AGA CTG TTG TTT GCG GGC 3'	(SEQ. ID NO. 131)	5' TAG CAC GAC AGT TGT ATG CAT G 3'	(SEQ. ID NO. 132)	128	CT	60 °C
WMS192	5' GGT TTT CTT TCA GAT TGC GC 3'	(SEQ. ID NO. 133)	5' CGT TGT CTA ATC TTG CCT TGC 3'	(SEQ. ID NO. 134)	191	CT	60 °C
WMS193	5' CTT TGT GCA CCT CTC TCT CC 3'	(SEQ. ID NO. 135)	5' AAT TGT GTT GAT GAT TTG GGG 3'	(SEQ. ID NO. 136)	171	CT,CA	60 °C
WMS194	5' GAT CTG CTC TAC TCT CCT CC 3'	(SEQ. ID NO. 137)	5' CGA CGC AGA ACT TAA ACA AG 3'	(SEQ. ID NO. 138)	131	CT	50 °C
WMS195	5' AGG TGC CGT CGC GTC TAC 3'	(SEQ. ID NO. 139)	5' ACC CCC CAC GTC AGA GAG 3'	(SEQ. ID NO. 140)	108	CT	60 °C
WMS197	5' GAG AAA GAG GTC TGG AGG TCG 3'	(SEQ. ID NO. 141)	5' CAA AAT GCA CAA GAA TGG AGG 3'	(SEQ. ID NO. 142)	126	CT	60 °C
WMS198	5' TTG AAC CGG AAG GAG TAC AG 3'	(SEQ. ID NO. 143)	5' TCA GTT TAT TTT GGG CAT GTG 3'	(SEQ. ID NO. 144)	130	CA	60 °C
WMS200	5' TCA ACG GAA CAG AGC AGC G 3'	(SEQ. ID NO. 145)	5' GAC CTG ATG AGA GCA AGC AC 3'	(SEQ. ID NO. 146)	250	CT	60 °C
WMS203	5' CCC AAA GCA GCG CAA GC 3'	(SEQ. ID NO. 147)	5' ACC AAT GCT ATC GGC TCG 3'	(SEQ. ID NO. 148)	139	CA,GA	55 °C
WMS205	5' CGA CCC GGT TCA CTT CAG 3'	(SEQ. ID NO. 149)	5' AGT CGC CGT TGT ATA GTG CC 3'	(SEQ. ID NO. 150)	152	CT	60 °C
WMS210	5' TGC ATC AAG AAT AGT GTG GAA G 3'	(SEQ. ID NO. 151)	5' TGA GAG GAA GGC TCA CAC CT 3'	(SEQ. ID NO. 152)	192	GA	60 °C
WMS212	5' AAG CAA CAT TTG CTG CAA TG 3'	(SEQ. ID NO. 153)	5' TGC AGT TAA CTT GTT GAA AGG A 3'	(SEQ. ID NO. 154)	104	CT	60 °C
WMS213	5' TGC CTG GCT GCT GTT TCT ATC TC 3'	(SEQ. ID NO. 155)	5' CTA GCT TAG CAC TGT CGC CC 3'	(SEQ. ID NO. 156)	184	GA	60 °C
WMS218	5' CGG CAA ACG GAT ATC GAC 3'	(SEQ. ID NO. 157)	5' AAC AGT AAC TCT CGC CAT AGC C 3'	(SEQ. ID NO. 158)	149	CT	60 °C
WMS219	5' GAT GAG CGA CGA CAC CTA GCC TC 3'	(SEQ. ID NO. 159)	5' GGG GTC CGA GTC CAC AAC 3'	(SEQ. ID NO. 160)	181	GAimp	60 °C
WMS224	5' TGA GTC CAG CAC CAC TGC 3'	(SEQ. ID NO. 161)	5' CAA CAT CCG CTC GTA TTC AA 3'	(SEQ. ID NO. 162)	142	CT	50 °C

Name	Forward primer	Reverse primer	Size	Allele	Temp.
WMS228	5' TCA TAT GCA CCT CTT TCC TAG G 3' (SEQ. ID NO. 163)	5' GTG TGC CAC CTT TGA CGT C 3' (SEQ. ID NO. 164)	210	CT,CA	60 °C
WMS231	5' AGC TCG GGA TGA AGC GTG 3' (SEQ. ID NO. 165)	5' GAT CCG CCG CTG CGT TT 3' (SEQ. ID NO. 166)	130	GAimp	60 °C
WMS232	5' ATC TCA ACG GCA AGC CG 3' (SEQ. ID NO. 167)	5' CTG ATG CAA GCA ATC CAC C 3' (SEQ. ID NO. 168)	141	GA	55 °C
WMS233	5' TCA AAA CAT AAA TGT TCA TTG GA 3' (SEQ. ID NO. 169)	5' TCA ACC GTG TGT AAT TTT GTC C 3' (SEQ. ID NO. 170)	261	CT	60 °C
WMS234	5' GAGTCC TGA TGT GAA GCT GTT G 3' (SEQ. ID NO. 171)	5' CTC ATT GGG GTG TGT ACG TG 3' (SEQ. ID NO. 172)	241	CT,CA	55 °C
WMS237	5' GAA TCA CTT GTG AAG CAT CTG G 3' (SEQ. ID NO. 173)	5' CTG GAT GCA TCA CAT CCA AC 3' (SEQ. ID NO. 174)	137	CT	55 °C
WMS238	5' TCG CTT CTA CCG CTC ACC 3' (SEQ. ID NO. 175)	5' AGT GCC TTG CCG AGG TC 3' (SEQ. ID NO. 176)	204	CT,GT,GGGT	55 °C
WMS241	5' TCT TCC AAC TAA AGC ATA GC 3' (SEQ. ID NO. 177)	5' CTT CCA TGG ACT ACA TAC TAG C 3' (SEQ. ID NO. 178)	146	GA	55 °C
WMS242	5' TCC AAG GCA GTA GGC AGG 3' (SEQ. ID NO. 179)	5' TGT TGT TGG CCT GTA TGC AT 3' (SEQ. ID NO. 180)	142	GA	55 °C
WMS244	5' GGC AGC TGA GGC AAT CTG 3' (SEQ. ID NO. 181)	5' TTT GGA CAT TTC CCA GCG 3' (SEQ. ID NO. 182)	227	CAimp	60 °C
WMS245	5' CAG CGC AGT TAG CTC GC 3' (SEQ. ID NO. 183)	5' ATC TGT ACA TTC GAG CGC 3' (SEQ. ID NO. 184)	141	CT	60 °C
WMS247	5' GCA ATC TTT TTT CTG ACC ACG 3' (SEQ. ID NO. 185)	5' ATG TGC ATG TCG GAC GC 3' (SEQ. ID NO. 186)	158	GA	60 °C
WMS248	5' AGG ACT TCC GCA CCC TG 3' (SEQ. ID NO. 187)	5' TGG CGT GGT CTA AAT GGA C 3' (SEQ. ID NO. 188)	185	CA	60 °C
WMS249	5' CAA ATG GAT CGA GAA AGG GA 3' (SEQ. ID NO. 189)	5' CTG CCA TTT TTC TGG ATC TAC C 3' (SEQ. ID NO. 190)	177	GAimp	60 °C
WMS251	5' CAA CTG GTT GCT ACA CAA GCA 3' (SEQ. ID NO. 191)	5' GGG ATG TCT GTT CCA TCT TAG 3' (SEQ. ID NO. 192)	103	CA	55 °C
WMS255	5' CAA CTG TAC GTA GGT TTC ATT GC 3' (SEQ. ID NO. 193)	5' TCT GCC GTA AGT CGC CTC 3' (SEQ. ID NO. 194)	148	GA	55 °C
WMS257	5' AGA GTG CAT GGT GGG ACG 3' (SEQ. ID NO. 195)	5' CCA AGA CGA TGC TGA AGT CA 3' (SEQ. ID NO. 196)	192	GT	60 °C
WMS258	5' GAT CGC TTC ATC TCT CTC TCT C 3' (SEQ. ID NO. 197)	5' GTA CAC GCC GTA GGC CC 3' (SEQ. ID NO. 198)	>81	CT	60 °C
WMS259	5' AGG GAA AAG ACA TCT TTT TTT TC 3' (SEQ. ID NO. 199)	5' CGA CCG ACT TCG GGT TC 3' (SEQ. ID NO. 200)	105	GA	55 °C
WMS260	5' GCC CCC TTG CAC AAA TC 3' (SEQ. ID NO. 201)	5' CGC AGC TAC AGG AGG CC 3' (SEQ. ID NO. 202)	157	GA	55 °C
WMS261	5' CTC CCT GTA CGC CTA AGG C 3' (SEQ. ID NO. 203)	5' CTC GCG CTA CTA GCC ATT G 3' (SEQ. ID NO. 204)	192	CT	55 °C
WMS263	5' TCT GCC GTA AGT CGC CTC 3' (SEQ. ID NO. 205)	5' GGT TTC ATT GCT TGC CCT AA 3' (SEQ. ID NO. 206)	134	CT	55 °C
WMS264	5' GAG AAA CAT GCC GAA CAA CA 3' (SEQ. ID NO. 207)	5' GCA TGC ATG AGA ATA GGA ACT G 3' (SEQ. ID NO. 208)	219	CA	60 °C
WMS265	5' TGT TGC GGA TGG TCA CTA TT 3' (SEQ. ID NO. 209)	5' GAG TAC ACA TTT GGC CTC TGC 3' (SEQ. ID NO. 210)	200	GT	55 °C
WMS268	5' AGG GGA TAT GTT GTC ACT CCA 3' (SEQ. ID NO. 211)	5' TTA TGT GAT TGC GTA CGT ACC C 3' (SEQ. ID NO. 212)	241	GAimp	55 °C
WMS269	5' TGC ATA TAA ACA GTC ACA CAC CC 3' (SEQ. ID NO. 213)	5' TTT GAG CTC CAA AGT GAG TTA GC 3' (SEQ. ID NO. 214)	>148	CA	60 °C
WMS271	5' CAA GAT CGT GGA GCC AGC 3' (SEQ. ID NO. 215)	5' AGC TGC TAG CTT TTG GGA CA 3' (SEQ. ID NO. 216)	162	CT,GA	60 °C
WMS272	5' TGC TCT TTG GCG AAT ATA TGG 3' (SEQ. ID NO. 217)	5' GTT CAA AAC AAA TTA AAA GGC CC 3' (SEQ. ID NO. 218)	140	CA	55 °C

	Primer sequence 1	Primer sequence 2	Size		Temp
WMS273	5' ATT GGA CGG ACA GAT GCT TT 3' (SEQ. ID NO. 219)	5' AGC AGT GAG GAA GGG GAT C 3' (SEQ. ID NO. 220)	167	GA	55 °C
WMS274	5' AAC TTG CAA AAC TGT TCT GA 3' (SEQ. ID NO. 221)	5' TAT TTG AAG CGG TTT GAT TT 3' (SEQ. ID NO. 222)	179	GT	50 °C
WMS275	5' AAT TTT CTT CCT CAC TTA TTC T 3' (SEQ. ID NO. 223)	5' AAC AAA AAA TTA GGG CC 3' (SEQ. ID NO. 224)	107	CT	50 °C
WMS276	5' ATT TGC CTG AAG AAA ATA TT 3' (SEQ. ID NO. 225)	5' AAT TTC ACT GCA TAC ACA AG 3' (SEQ. ID NO. 226)	99	CT	55 °C
WMS278	5' GTT GCT TCA TGA ACG CTC AA 3' (SEQ. ID NO. 227)	5' CTG CCC AAT TTT CTC CAC TC 3' (SEQ. ID NO. 228)	241	GTimpGAimp	55 °C
WMS281	5' CGG CCA TAT TTC TGT AAG TAT GC 3' (SEQ. ID NO. 229)	5' GCA GGT AAT GGC CGG AC 3' (SEQ. ID NO. 230)	135	GT	60 °C
WMS282	5' TTG GCC GTG TAA GGC AG 3' (SEQ. ID NO. 231)	5' TCT CAT TCA CAC ACA ACA CTA GC 3' (SEQ. ID NO. 232)	220	GA	55 °C
WMS284	5' AAT GAA AAA ACA CTT GCG TGG 3' (SEQ. ID NO. 233)	5' GCA CAT TTT TCA CTT TCG GG 3' (SEQ. ID NO. 234)	123	GA	60 °C
WMS285	5' ATG ACC CTT CTG CCA AAC AC 3' (SEQ. ID NO. 235)	5' ATC GAC CGG GAT CTA GCC 3' (SEQ. ID NO. 236)	243	GA	60 °C
WMS291	5' CAT CCC TAC GCC ACT CTG C 3' (SEQ. ID NO. 237)	5' AAT GGT ATC TAT TCC GAC CCG 3' (SEQ. ID NO. 238)	>158	CA	60 °C
WMS292	5' TCA CCG TGG TCA CCG AC 3' (SEQ. ID NO. 239)	5' CCA CCG.AGC CGA TAA TGT AC 3' (SEQ. ID NO. 240)	220	CT	60 °C
WMS293	5' TAC TGG TTC ACA TTG GTG TGG 3' (SEQ. ID NO. 241)	5' TCG CCA TCA CTC GTT CAA G 3' (SEQ. ID NO. 242)	201	CA	55 °C
WMS294	5' GGA TTG GAG TTA AGA GAG AAC CG 3' (SEQ. ID NO. 243)	5' GCA GAG TGA TCA ATG CCA GA 3' (SEQ. ID NO. 244)	100	GAimp	55 °C
WMS295	5' GTG AAG CAG ACC ACC CAC AC 3' (SEQ. ID NO. 245)	5' GAC GGC TGC GAC GTA GAG 3' (SEQ. ID NO. 246)	258	GA	60 °C
WMS296	5' AAT TCA ACC TAC CAA TCT CTG 3' (SEQ. ID NO. 247)	5' GCC TAA TAA ACT GAA AAC GAG 3' (SEQ. ID NO. 248)	149	CT	55 °C
WMS297	5' ATC GTC ACG TAT TTT GCA ATG 3' (SEQ. ID NO. 249)	5' TGC GTA AGT CTA GCA TTT TCT G 3' (SEQ. ID NO. 250)	150	GT, GA	55 °C
WMS299	5' ACT ACT TAG GCC TCC CGC C 3' (SEQ. ID NO. 251)	5' TGA CCC ACT TGC AAT TCA TC 3' (SEQ. ID NO. 252)	208	GA, TAG	55 °C
WMS301	5' GAG GAG TAA GAC ACA TGC CC 3' (SEQ. ID NO. 253)	5' GTG GCT GGA GAT TCA GGT TC 3' (SEQ. ID NO. 254)	204	GA,G	55 °C
WMS302	5' GCA AGA AGA AAC AAC AGC AGT AAC 3' (SEQ. ID NO. 255)	5' CAG ATG CTC TTC TCT GCT GG 3' (SEQ. ID NO. 256)	180 (340)	GA	60 °C
WMS304	5' AGG AAA CAG AAA TAT CGC GG 3' (SEQ. ID NO. 257)	5' AGG ACT GTG GGG AAT GAA TG 3' (SEQ. ID NO. 258)	217	CT	55 °C
WMS311	5' TCA CGT GGA AGA CGC TCC 3' (SEQ. ID NO. 259)	5' CTA CGT GCA CCA TTT TG 3' (SEQ. ID NO. 260)	151	GA	60 °C
WMS312	5' ATC GCA TGA TGC ACG TAG AG 3' (SEQ. ID NO. 261)	5' ACA TGC ATG CCT ACC TAA TGG 3' (SEQ. ID NO. 262)	235	GA	60 °C
WMS313	5' CCG CCC TCA TTA AGT TTC AC 3' (SEQ. ID NO. 263)	5' TTT GAC AAG TAC ACG AGT CTG C 3' (SEQ. ID NO. 264)	156	CT,GT	55 °C
WMS314	5' AGG AGC TCC TCT GTG CCA C 3' (SEQ. ID NO. 265)	5' TTC GGG ACT CTC TTC CCT G 3' (SEQ. ID NO. 266)	170	CT	55 °C
WMS316	5' CAT GGA CAT TTT ACC ACA AGA C 3' (SEQ. ID NO. 267)	5' TGC GTG TGG TCC ACC TC 3' (SEQ. ID NO. 268)	176	AT,GT	55 °C
WMS319	5' GGT TGC TGT ACA AGT GTT CAC G 3' (SEQ. ID NO. 269)	5' CGG GTG CTG CTG TGT GTA ATG AC 3' (SEQ. ID NO. 270)	200	CT	55 °C
WMS320	5' CGA GAT ACT ATG GAC GGT GAG G 3' (SEQ. ID NO. 271)	5' ATC TTT GCA AGG ATT GCC C 3' (SEQ. ID NO. 272)	>263	GT,GA	55 °C
WMS321	5' CAA TGT GGA GAC GGT GTG C 3' (SEQ. ID NO. 273)	5' TGT TGC ATG CGA TCA TGC 3' (SEQ. ID NO. 274)	265	GT,GAimp	60 °C

Name	Primer 1		Primer 2		No.	Code	Temp.
WMS322	5'TCA CAA AAT GAT TTC TCA TCC G 3'	(SEQ. ID NO. 275)	5'TGC AGA AAA CCA ACA AGG G 3'	(SEQ. ID NO. 276)	119	GA	55°C
WMS325	5'TTT CTT CTG TCG TTC TCT TCC C 3'	(SEQ. ID NO. 277)	5'TTT TTA CGC GTC AAC GAC G 3'	(SEQ. ID NO. 278)	131	CT	55°C
WMS328	5'GCA ATC CAC GAG AAG AGA GG 3'	(SEQ. ID NO. 279)	5'CAC AAA CTC TTG ACA TGT GCG 3'	(SEQ. ID NO. 280)	193	GT	55°C
WMS330	5'TTG CTA TCC ATG TGC CAG AG3'	(SEQ. ID NO. 281)	5'ACA TGT TTC ATG CAG GTA GCC 3'	(SEQ. ID NO. 282)	165	GTT	55°C
WMS332	5'AGC CAG CAA GTC ACC AAA AC 3'	(SEQ. ID NO. 283)	5'AGT GCT GGA AAG AGT AGT GAA GC 3'	(SEQ. ID NO. 284)	231	GA	60°C
WMS333	5'GCC CGG TCA TGT AAA ACG 3'	(SEQ. ID NO. 285)	5'TTT CAG TTT GCG TTA AGC TTT G 3'	(SEQ. ID NO. 286)	150	GA	55°C
WMS334	5'AAT TTC AAA AAG GAG AGA GA 3'	(SEQ. ID NO. 287)	5'AAC ATG TGT TTT TAG CTA TC 3'	(SEQ. ID NO. 288)	123	GA	50°C
WMS335	5'CGT ACT CCA CTC CAC ACG G 3'	(SEQ. ID NO. 289)	5'CGG TCC AAG TGC TAC CTT TC 3'	(SEQ. ID NO. 290)	187 (225)	GA,GCGT	55°C
WMS336	5'CCC TTT AAT CTC GCT CCC TC 3'	(SEQ. ID NO. 291)	5'GTC TCT TTC TCG TAC TTC CAG G 3'	(SEQ. ID NO. 292)	108	CT	55°C
WMS337	5'CCT CTT CCT CCC TCA CTT AGC 3'	(SEQ. ID NO. 293)	5'TGC TAA CTG GCC TTT GCC 3'	(SEQ. ID NO. 294)	183	CT,CACT,CA	55°C
WMS339	5'AAT TTT CTT CTT CAC TTA TT 3'	(SEQ. ID NO. 295)	5'AAA CGA ACA ACC ACT CAA TC 3'	(SEQ. ID NO. 296)	159	CT	50°C
WMS340	5'GCA ATC TTT TTT CTG ACC ACG 3'	(SEQ. ID NO. 297)	5'ACG AGG CAA GAA CAC ACA TG 3'	(SEQ. ID NO. 298)	132	GA	60°C
WMS341	5'TTC AGT GGT AGC GGT CGA G 3'	(SEQ. ID NO. 299)	5'CCG ACA TCT CAT GGA TCC AC 3'	(SEQ. ID NO. 300)	133 (150)	CT	55°C
WMS342	5'TAT CCA GAG CAG ACG GAC CT 3'	(SEQ. ID NO. 301)	5'GGT CTA GCT TCG ACG ACA CC 3'	(SEQ. ID NO. 302)	169	GT	55°C
WMS344	5'CAA GGA AAT AGG CGG TAA CT 3'	(SEQ. ID NO. 303)	5'ATT TGA GTC TGA AGT TTG CA 3'	(SEQ. ID NO. 304)	131	GT	55°C
WMS346	5'CAA GCA AGG TTT CGT TTT ATC C 3'	(SEQ. ID NO. 305)	5'GCA TGT GGT CCA TGT ACT GC 3'	(SEQ. ID NO. 306)	203	AT,GT	55°C
WMS349	5'GGC TTC CAG AAA ACA ACA GG 3'	(SEQ. ID NO. 307)	5'ATC GGT GCG TAC CAT CCT AC 3'	(SEQ. ID NO. 308)	230	GA	55°C
WMS350	5'ACC TCA TCC ACA TGT TCT ACG 3'	(SEQ. ID NO. 309)	5'GCA TGG ATA GGA CGC CC 3'	(SEQ. ID NO. 310)	146	GT	60°C
WMS353	5'CCA TGT TGA GTA GGT TCA GCC 3'	(SEQ. ID NO. 311)	5'CTT GGC CAG AAG CTA CGA AC 3'	(SEQ. ID NO. 312)	179	GCGT,GT	55°C
WMS356	5'AGC GTT CTT GGG AAT TAG AGA 3'	(SEQ. ID NO. 313)	5'CCA ATC AGC CTG CAA CAA C 3'	(SEQ. ID NO. 314)	224	GA	55°C
WMS357	5'TAT GGT CAA AGT TGG ACC TCG 3'	(SEQ. ID NO. 315)	5'AGG CTG CAG CTC TTC TTC AG 3'	(SEQ. ID NO. 316)	123	GA	55°C
WMS358	5'AAA CAG CGG ATT TCA TCG AG 3'	(SEQ. ID NO. 317)	5'TCC GCT GTT GTT CTG ATC TC 3'	(SEQ. ID NO. 318)	164	GAimp	55°C
WMS359	5'CTA ATT GCA ACA GGT CAT GGG 3'	(SEQ. ID NO. 319)	5'TAC TTG TGT TCT GGG ACA ATG G 3'	(SEQ. ID NO. 320)	217	CT,CTTimp	60°C
WMS361	5'GTA ACT TGT TGC CAA AGG GG 3'	(SEQ. ID NO. 321)	5'ACA AAG TGG CAA AAG GAG ACA 3'	(SEQ. ID NO. 322)	126	GAimp	60°C
WMS368	5'CCA TTT CAC CTA ATG CCT GC 3'	(SEQ. ID NO. 323)	5'AAT AAA ACC ATG AGC TCA CTT GC 3'	(SEQ. ID NO. 324)	249	AT	60°C
WMS369	5'CTG CAG GCC ATC ATG ATG ATG 3'	(SEQ. ID NO. 325)	5'ACC GTG GGT GTT GTG AGC 3'	(SEQ. ID NO. 326)	188	CTimp	60°C
WMS371	5'GAC CAA GAT ATT CAA ACT GGC C 3'	(SEQ. ID NO. 327)	5'AGC TCA GCT TGC TTG GTA CC 3'	(SEQ. ID NO. 328)	170	CA, GA	60°C
WMS372	5'AAT AGA GCC CTG GGA CTG GG 3'	(SEQ. ID NO. 329)	5'GAA GGA CGA CAT TCC ACC TG 3'	(SEQ. ID NO. 330)	>329	GA	60°C

	Forward primer	SEQ ID	Reverse primer	SEQ ID	Size	Repeat	Temp
WMS374	5'ATA GTG TGT TGC ATG CTG TGT G 3'	(SEQ. ID NO. 331)	5'TCT AAT TAG CGT TGG CTG CC 3'	(SEQ. ID NO. 332)	213	GT	60 °C
WMS375	5'ATT GGC GAC TCT AGC ATA TAC G 3'	(SEQ. ID NO. 333)	5'GGG ATG TCT GTT CCA TCT TAG C 3'	(SEQ. ID NO. 334)	156	CA	55 °C
WMS376	5'GGG CTA GAA AAC AGG AAG GC 3'	(SEQ. ID NO. 335)	5'TCT CCC GGA GGG TAG GAG 3'	(SEQ. ID NO. 336)	147	CA, GAimp	60 °C
WMS382	5'GTC AGA TAA CGC CGT CCA AT 3'	(SEQ. ID NO. 337)	5'CTA CGT GCA CCA CCA TTT TG 3'	(SEQ. ID NO. 338)	115	GA	60 °C
WMS383	5'ACG CCA GTT GAT CCG TAA AC 3'	(SEQ. ID NO. 339)	5'GAC ATC AAT AAC CGT GGA TGG 3'	(SEQ. ID NO. 340)	195	GT	60 °C
WMS384	5'TTT TCA TTG TGC CCT CTA CT 3'	(SEQ. ID NO. 341)	5'GCC AAG TTT CTT AGC TAG TTA A 3'	(SEQ. ID NO. 342)	204	GTimp	55 °C
WMS388	5'CTA CAA TTC GAA GGA GAG GGG 3'	(SEQ. ID NO. 343)	5'CAC CGC GTC AACTAC TTA AGC 3'	(SEQ. ID NO. 344)	162	CT,CA,CA	60 °C
WMS389	5'ATC ATG TCG ATC TCC TTG ACG 3'	(SEQ. ID NO. 345)	5'TGC CAT GCA CAT TAG CAG AT 3'	(SEQ. ID NO. 346)	130	CT,GT	60 °C
WMS390	5'AAG TTT CAC ACA AGA TCT CTC C 3'	(SEQ. ID NO. 347)	5'TGA CAA GTA CAC GAG TCT GC 3'	(SEQ. ID NO. 348)	143	CT,GT	55 °C
WMS391	5'ATA GCG AAG TCT CCC TAC TCC A 3'	(SEQ. ID NO. 349)	5'ATG TGC ATG TCG GAC GC 3'	(SEQ. ID NO. 350)	150	CA,GA	55 °C
WMS393	5'TCA TCT GCT ATT TGT GCT ACA 3'	(SEQ. ID NO. 351)	5'TCA AAT ACA CCA ATG TGC C 3'	(SEQ. ID NO. 352)	107	CA	55 °C
WMS395	5'TAC AAC CGC AAG TAA TGC CA 3'	(SEQ. ID NO. 353)	5'TAC CAA CAC CCT AGC CCT TG 3'	(SEQ. ID NO. 354)	147	CA	60 °C
WMS397	5'TGT CAT GGA TTA TTT GGT CGG 3'	(SEQ. ID NO. 355)	5'CTG CAC TCT CGG TAT ACC AGC 3'	(SEQ. ID NO. 356)	179	CT	55 °C
WMS400	5'GTG CTG CCA CCA CTT GC 3'	(SEQ. ID NO. 357)	5'TGT AGG CAC TGC TTG GGA G 3'	(SEQ. ID NO. 358)	139	CA	60 °C
WMS403	5'CGA CAT TGG CTT CGG TG 3'	(SEQ. ID NO. 359)	5'ATA AAA CAG TGC GGT CCA GG 3'	(SEQ. ID NO. 360)	133	CA	55 °C
WMS408	5'TCG ATT TAT TTG GGC CAC AGT 3'	(SEQ. ID NO. 361)	5'GTA TAA TTC GTT CAC AGC ACG C 3'	(SEQ. ID NO. 362)	176	CA	55 °C
WMS410	5'GCT TGA GAC CGG CAC AGT 3'	(SEQ. ID NO. 363)	5'CGA GAC CTT GAG GGT CTA GA 3'	(SEQ. ID NO. 364)	334	CA	55 °C
WMS411	5'CCC ATA CGA TGA TGT GTT TCC 3'	(SEQ. ID NO. 365)	5'CAA ACG GAA CAT GGT CCC 3'	(SEQ. ID NO. 366)	148	CT	55 °C
WMS412	5'ATC AAC AAG GTT TGT GTG TTG G 3'	(SEQ. ID NO. 367)	5'ATG AAA CGC GAC CTC CC 3'	(SEQ. ID NO. 368)	121	GA	60 °C
WMS413	5'TGC TTG TGT TCT AGA TTG CTT GGG 3'	(SEQ. ID NO. 369)	5'GAT CGT CTC GTC CTT GGC A 3'	(SEQ. ID NO. 370)	94	GA	55 °C
WMS415	5'GAT CTC CCA TGT CCG CC 3'	(SEQ. ID NO. 371)	5'CGA CAG TCG TCA CTT GCC TA 3'	(SEQ. ID NO. 372)	131	GAimp	60 °C
WMS425	5'GAG CCC ACA AGC TGG CA 3'	(SEQ. ID NO. 373)	5'TCG TTC TCC CAA GGC TTG 3'	(SEQ. ID NO. 374)	>143	CT	50 °C
WMS427	5'AAA CTT AGA ACT GTA ATT TCA GA 3'	(SEQ. ID NO. 375)	5'AGT GTG TTC ATT TGA CAG TT 3'	(SEQ. ID NO. 376)	215	CA	60 °C
WMS428	5'CGA GGC AGC GAG GAT TT 3'	(SEQ. ID NO. 377)	5'TTC TCC ACT AGC CCC GC 3'	(SEQ. ID NO. 378)	143	GA	50 °C
WMS429	5'TTG TAC ATT AAG TTC CCA TTA 3'	(SEQ. ID NO. 379)	5'TTT AAG GAC CTA CAT GAC AC 3'	(SEQ. ID NO. 380)	221 (290)	CT	55 °C
WMS434	5'ATG AGT TCC GCC AAA GAA TG 3'	(SEQ. ID NO. 381)	5'ACG AAA TAC ACA AGT GGG ACA 3'	(SEQ. ID NO. 382)	216	GT	50 °C
WMS437	5'GAT CAA GAC TTT TGT ATC TCT C 3'	(SEQ. ID NO. 383)	5'GAT GTC CAA CAG TTA GCT TA 3'	(SEQ. ID NO. 384)	109	CT	50 °C
WMS440	5'CCT ATG GTC TCC ATC ATG AGG 3'	(SEQ. ID NO. 385)	5'TCA TGT CAA CTC AAG AAC ACG 3'	(SEQ. ID NO. 386)	112	CT	55 °C

Marker	Forward Primer	SEQ ID NO	Reverse Primer	SEQ ID NO	Size	Repeat	Temp
WMS443	5'GGG TCT TCA TCC GGA ACT CT 3'	(SEQ. ID NO. 387)	5'CCA TGA TTT ATA AAT TCC ACC 3'	(SEQ. ID NO. 388)	134	CA,GA	55 °C
WMS445	5'TTT GTT GGG GGT TAG GAT TAG 3'	(SEQ. ID NO. 389)	5'CCT TAA CAC TTG CTG GTA GTG A 3'	(SEQ. ID NO. 390)	192	CT	55 °C
WMS448	5'AAA CCA TAT TGG GAG GAA AGG 3'	(SEQ. ID NO. 391)	5'CAC ATG GCA TCA CAT TTG TG 3'	(SEQ. ID NO. 392)	231	GA	60 °C
WMS455	5'ATT CGG TTC GCT AGC TAC CA 3'	(SEQ. ID NO. 393)	5'ACG GAG AGC AAC CTG CC 3'	(SEQ. ID NO. 394)	151	GTimp	55 °C
WMS456	5'TCT GAA CAT TAC ACA ACC CTG A 3'	(SEQ. ID NO. 395)	5'TGC TCT CTC TGA ACC TGA AGC 3'	(SEQ. ID NO. 396)	132	GA	55 °C
WMS458	5'AAT GGC AAT GGG AAG ACA TAG C 3'	(SEQ. ID NO. 397)	5'TTC GCA ATG TTG ATT TGG C 3'	(SEQ. ID NO. 398)	113	CA	60 °C
WMS459	5'ATG GAG TGG TCA CACTTT GAA 3'	(SEQ. ID NO. 399)	5'AGC TTC TCT GAC CAA CTT CTC G 3'	(SEQ. ID NO. 400)	>138	GA	55 °C
WMS469	5'CAA CTC AGT GCT CAC ACA ACG 3'	(SEQ. ID NO. 401)	5'CGA TAA CCA CTC ATC CAC ACC 3'	(SEQ. ID NO. 402)	>156	CT	60 °C
WMS471	5'CGG CCC TAT CAT GGC TG 3'	(SEQ. ID NO. 403)	5'GCT TGC AAG TTC CAT TTT GC 3'	(SEQ. ID NO. 404)	149	CA	55 °C
WMS473	5'TCA TAC GGG TAT GGT TGG AC 3'	(SEQ. ID NO. 405)	5'CAC CCC CTT GTT GGT CAC 3'	(SEQ. ID NO. 406)	220	GTimp	60 °C
WMS476	5'ATG GGT TCG TAC TAA CAT CAG C 3'	(SEQ. ID NO. 407)	5'TTG CTG GTA GCT TCA ATC CC 3'	(SEQ. ID NO. 408)	>194	GAimp	60 °C
WMS480	5'TGC TGC TAC TTG TAC AGA GGA C 3'	(SEQ. ID NO. 409)	5'CCG AAT TGT CCG CCA TAG 3'	(SEQ. ID NO. 410)	188	CT, CA	55 °C
WMS484	5'ACA TCG CTC TTC ACA AAC CC 3'	(SEQ. ID NO. 411)	5'AGT TCC GGT CAT GGC TAG G 3'	(SEQ. ID NO. 412)	145	CT	60 °C
WMS494	5'ATT GAA CAG GAA GAC ATC AGG G 3'	(SEQ. ID NO. 413)	5'TTC CTG GAG CTG TGT GGC 3'	(SEQ. ID NO. 414)	198	CA	60 °C
WMS495	5'GAG AGC CTC GCG AAA TAT AGG 3'	(SEQ. ID NO. 415)	5'TGC TTC TGG TGT TCC TTC G 3'	(SEQ. ID NO. 416)	168	GA	55 °C
WMS497	5'GTA GTG AAG ACA AGG GCA TT 3'	(SEQ. ID NO. 417)	5'CCG AAA GTT GGG TGA TAT AC 3'	(SEQ. ID NO. 418)	>106	GTimp	60 °C
WMS499	5'ACT TGT ATG CTC CAT TGA TTG G 3'	(SEQ. ID NO. 419)	5'GGG GAG TGG AAA CTG CAT AA 3'	(SEQ. ID NO. 420)	145	GA	60 °C
WMS501	5'GGC TAT CTC TGG CGC TAA AA 3'	(SEQ. ID NO. 421)	5'TCC ACA AAC AAG TAG CGC C 3'	(SEQ. ID NO. 422)	172	CA	60 °C
WMS512	5'AGC CAC CAT CAG CAA AAT T 3'	(SEQ. ID NO. 423)	5'GAA CAT CTT GTTC ATG GCA CAT TG 3'	(SEQ. ID NO. 424)	185	GT	60 °C
WMS513	5'ATC CGT AGC ACC TAC TGG TCA 3'	(SEQ. ID NO. 425)	5'GGT CTT GTTC ATG GTG TGC CTC A 3'	(SEQ. ID NO. 426)	144	CA	55 °C
WMS515	5'AAC ACA ATG GCA AAT GCG TGA 3'	(SEQ. ID NO. 427)	5'CCT TCC TAG TAA GTG TGC CTC A 3'	(SEQ. ID NO. 428)	134	GTimp	55 °C
WMS518	5'AAT CAC AAC AAG GCG TGA CA 3'	(SEQ. ID NO. 429)	5'CAG GGT GGT GCA TGC AT 3'	(SEQ. ID NO. 430)	166	CA	60 °C
WMS530	5'AAA TAG GAC AAC CCA CGG C 3'	(SEQ. ID NO. 431)	5'TCA ACT TCT TGG CCT CCA TC 3'	(SEQ. ID NO. 432)	186	CT	60 °C
WMS532	5'ACT GCG TGT GCC TAC AAT TG 3'	(SEQ. ID NO. 433)	5'TCA CTC GCA CTC GAT AGG C 3'	(SEQ. ID NO. 434)	142	GT	60 °C
WMS533	5'AAG GCG AAT CAA ACG GAA TA 3'	(SEQ. ID NO. 435)	5'GTT GCT TTA GGG GAA AAG CC 3'	(SEQ. ID NO. 436)	147	CT, CA	60 °C
WMS537	5'ACA TAA TGC TTC CTG TGC ACC 3'	(SEQ. ID NO. 437)	5'GCC ACT TTT GTG TCG TTC CT 3'	(SEQ. ID NO. 438)	209	CA, TA	60 °C
WMS538	5'GCA TTT CGG GTG AAC CC 3'	(SEQ. ID NO. 439)	5'GTT GCA TGT ATA CGT TAA GCG G 3'	(SEQ. ID NO. 440)	147	GTimp	60 °C
WMS540	5'TCT CGC TGT GAA ATC CTA TTT C 3'	(SEQ. ID NO. 441)	5'AGG CAT GGA TAG AGG GGC 3'	(SEQ. ID NO. 442)	129	CTimp	55 °C

	Forward primer	Reverse primer	Size	Repeat	Temp
WMS544	5' TAG AAT TCT TTA TGG GGT CTG C 3' (SEQ. ID NO. 443)	5' AGG ATT CCA ATC CTT CAA AAT T 3' (SEQ. ID NO. 444)	167	CT,ATCT,CT	55 °C
WMS550	5' CCC ACA AGA ACC TTT GAA GA 3' (SEQ. ID NO. 445)	5' CAT TGT GTG TGC AAG GCA C 3' (SEQ. ID NO. 446)	150	CT,GT	55 °C
WMS554	5' TGC CCA CAA CGG AAC TTG 3' (SEQ. ID NO. 447)	5' GCA ACC ACC AAG CAC AAA GT 3' (SEQ. ID NO. 448)	160	CT,GTimp	60 °C
WMS565	5' GCG TCA GAT ATG CCT ACC TAG G 3' (SEQ. ID NO. 449)	5' AGT GAG TTA GCC CTG AGC CA 3' (SEQ. ID NO. 450)	142	CA	60 °C
WMS566	5' TCT GTC TAC CCA TGG GAT TTG 3' (SEQ. ID NO. 451)	5' CTG GCT TCG AGG TAA GCA AC 3' (SEQ. ID NO. 452)	130	CA,TA	60 °C
WMS569	5' GGA AAC TTA TTG ATT GAA AT 3' (SEQ. ID NO. 453)	5' TCA ATT TTG ACA GAA GAA TT 3' (SEQ. ID NO. 454)	134	GT	47 °C
WMS570	5' TCG CCT TTT ACA GTC GGC 3' (SEQ. ID NO. 455)	5' ATG GGT AGC TGA GAG CCA AA 3' (SEQ. ID NO. 456)	143	CT,GT	60 °C
WMS573	5' AAG AGA TAA CAT GCA AGA AA 3' (SEQ. ID NO. 457)	5' TTC AAA TAT GTG GGA ACT AC 3' (SEQ. ID NO. 458)	212	CA	50 °C
WMS577	5' ATG GCA TAA TTT GGT GAA ATT G 3' (SEQ. ID NO. 459)	5' TGT TTC AAG CCC AAC TTC TAT T 3' (SEQ. ID NO. 460)	133	CA,TA	55 °C
WMS582	5' AAG CAC TAC GAA AAT ATG AC 3' (SEQ. ID NO. 461)	5' TCT TAA GGG GTG TTA TCA TA 3' (SEQ. ID NO. 462)	151	CA	50 °C
WMS583	5' TTC ACA CCC AAC CAA TAG CA 3' (SEQ. ID NO. 463)	5' TCT AGG CAG ACA CAT GCC TG 3' (SEQ. ID NO. 464)	165	CA	60 °C
WMS588	5' GAT CCC CAA TTG CAT GTT G 3' (SEQ. ID NO. 465)	5' CTT GCA ACT GGG GGA CAC 3' (SEQ. ID NO. 466)	102	GT	60 °C

'CS' Weizensorte 'Chinese Spring'

These markers are distinguished by a high degree of polymorphism between different wheat varieties or lines and usually detect several alleles per genetic locus in different wheat lines.

They can therefore be used for DNA fingerprinting, species identification, relationship or similarity studies, characterization of cytological lines, such as deletion lines, substitution lines, addition lines, etc. and all forms of genetic mappings, including mapping of individual genes and quantitative distinguishing features (QTLs). In addition, their use is also very suitable for automation and it is possible to carry out the detection of the products with nonradioactive methods.

With the help of this inventive marker, the possibility is provided, for example, of differentiating almost all European wheat lines.

The invention is described in greater detail below by means of examples.

1. Amplification of the Microsatellite Markers

The microsatellite markers are amplified according to the following protocol:

10 mM tris-HCl, pH 8

50 mM KCl

1.5 mM $MgCl_2$ (in a few exceptional cases 3 mM $MgCl_2$)

0.01% (w/v) gelatin

0.2 mM of each desoxynucleotide

250 nM of each primer (in each case to the left and right of a pair)

1 - 2 units taq polymerase

50 - 150 ng matrixes (template) DNA

are amplified in a volume of 25 or 50 µL according to the following profile:

92°C   3 minute

92°C   1 minute (denaturing phase)

60°C   1 minutes (annealing phase)   45 cycles

72°C   2 minutes (elongation phase)

72°C   10 minutes (extension phase)

The amplification takes place in a Perkin Elmer 9600 with lid heating or in an MJ Research Thermocycler without lid heating. In this apparatus, a layer of mineral oil is placed over the reactions. The temperature of the annealing phase depends on the melting point (T_m) of the primer and in some cases even is 50°C or 55°C.

2.   Separation of the Microsatellite Markers on Polyacrylamide Gels, Which Are Not Denaturing

The PCR reactions are mixed with 1/10 volume of stop buffer (0.02 M tris acetate of pH 8.1, 0.025 M sodium acetate, 0.02 M EDTA, 70% glycerin, 0.2% SDS, 0.6% bromphenol blue, 0.6% xylene cyanol) and in each case 25 µL are separated in 10% polyacrylamide gels (1.5 mm thick, 18 cm long).

Formulation for polyacrylamide gel (10%):

25 mL   stock acrylamide solution (19 g acrylamide, 1 g bisacrylamide, diluted to 100 mL with water)

10 mL   5X TBE (1X TBE = 0.09 M tris borate of pH 8.3, 0.002 M EDTA

15 mL   water

are mixed and the polymerization is started by the addition of 220 µL of ammonium persulfate (10%, freshly prepared) and 20 µL of TEMED. Immediately after the addition, the mixture is poured into the sealed gel mold and the comb for forming pockets is inserted. The polymerization is completed after about 1 hour. The gel is placed in the gel chamber and a preliminary run is carried out without samples for about 30 minutes at 150 volts in 1X TBE. After that, the samples are loaded (25 µL of each) and the separation is carried out for 14 - 16 hours at 100 volts.

After the electrophoresis is completed, the gel is stained for about minutes in ethidium bromide (1 - 2 drops of 10 mg/mL in 1 liter of water) and the fragments are made visible by a UV transilluminator and documented.

3. Separation of Microsatellite Markers on Denaturing Gels

For the separation of the amplified fragments on denaturing gels, an automatic laser fluorescence (A.L.F.) sequencer (Pharmacia), for example, is used. In order to enable the fragments to be detected by means of a laser, one primer per pair is marked at the 5' end with fluorescein. Per PCR reaction, 0.3 to 1.5 microliters are mixed with 2.5 microliters of stop buffer (deionized formamide; 5 mg/mL dextran blue), denatured (1 minute; 90°C) and loaded onto the gel. Gel plates with a 9 cm separation distance are used, as recommended by the manufacturer for the fragment analysis. The gel solution contains 6.5% Long-Ranger (AT Biochem), 7M urea and 1.2X TBE buffer. The gels are 0.35 or 0.5 mm thick. The conditions for the gel run are 600 V, 40 mA, 50 W, 0.84 s data collection interval and 2 mW laser energy. The gel runs are ended after about 80 to 90 minuutes. This is sufficient for detecting fragments up to a size of 300 bp. A gel can be used for four or five runs. For each gel

run, a data set is obtained. With this data set and by means of internal size standards, the exact fragment sizes are determined in the computer program Fragment Manager (Pharmacia) and thus the smallest size differences of a base pair are determined.